Quantification of Toxic Effects for Water Concentration-Based Aquatic Life Criteria

Part B

Section 4: Pentachloroethane Lethality to Juvenile Fathead Minnows

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Section 4. Pentachloroethane Lethality To Juvenile Fathead Minnows

4.1 Overview

Erickson et al. (1991) conducted a series of experiments on the toxicity of pentachloroethane (PCE) to juvenile fathead minnows. These experiments included evaluations of bioaccumulation kinetics, the time-course of mortality under both constant and time-variable exposures, the response of fish growth rate to constant and time-variable exposures, and the relationship of toxic effects to PCE accumulation. This section will examine mortality in these experiments and evaluate the applicability of the toxicity models discussed in Section 2 of Part A of this report, first considering the "implicit" models already applied to copper toxicity in Section 3, and then the "explicit" models using information gathered in these experiments regarding PCE accumulation kinetics and the relationship of effects to PCE accumulation. Both deterministic and stochastic toxicity models will be evaluated, but only in their simplest forms (Equations 2.1-2.7, Equations 2.22-2.28), because the data in these experiments are insufficient to consider more complicated models with multiple processes and/or compartments. Subsequent work will evaluate growth effects in these experiments.

4.2 Study Description

The study of Erickson et al. (1991) consisted of the following experiments:

(1) A bioconcentration experiment (Experiment B1) in which ca. 4-week-old fathead minnows were exposed to five levels of PCE (ranging from 1 mg/L, a no effect concentration for growth and survival, to 10 mg/L, lethal within 12 h) for 48 h, followed by an elimination period of 24 h in uncontaminated water. Accumulation of PCE in the fish was monitored at 1, 2, 4, 8, 24, and 48 h during the exposure period and at 1, 2, 4, 8, and 24 h during the elimination period.

(2) 4-d tests of survival (Experiments A1, A2) with ca. 4-week-old fish. In Experiment A1, continuous exposures at five concentrations (Test A1c) were evaluated simultaneously with daily 6-h pulses of five intensities (Test A1p). In Experiment A2, continuous exposure (Test A2c) was contrasted both to daily 6-h pulses (Test A2p) and to an incrementally increasing ("stepped")

exposure (Test A2s), in which the five treatments started at the five same concentrations as the continuous exposure and each was increased daily to the next higher concentration (terminating each treatment after it reached the highest concentration for one day). Accumulation of PCE was measured in samples of fish that died and in fish surviving at the end of the test.

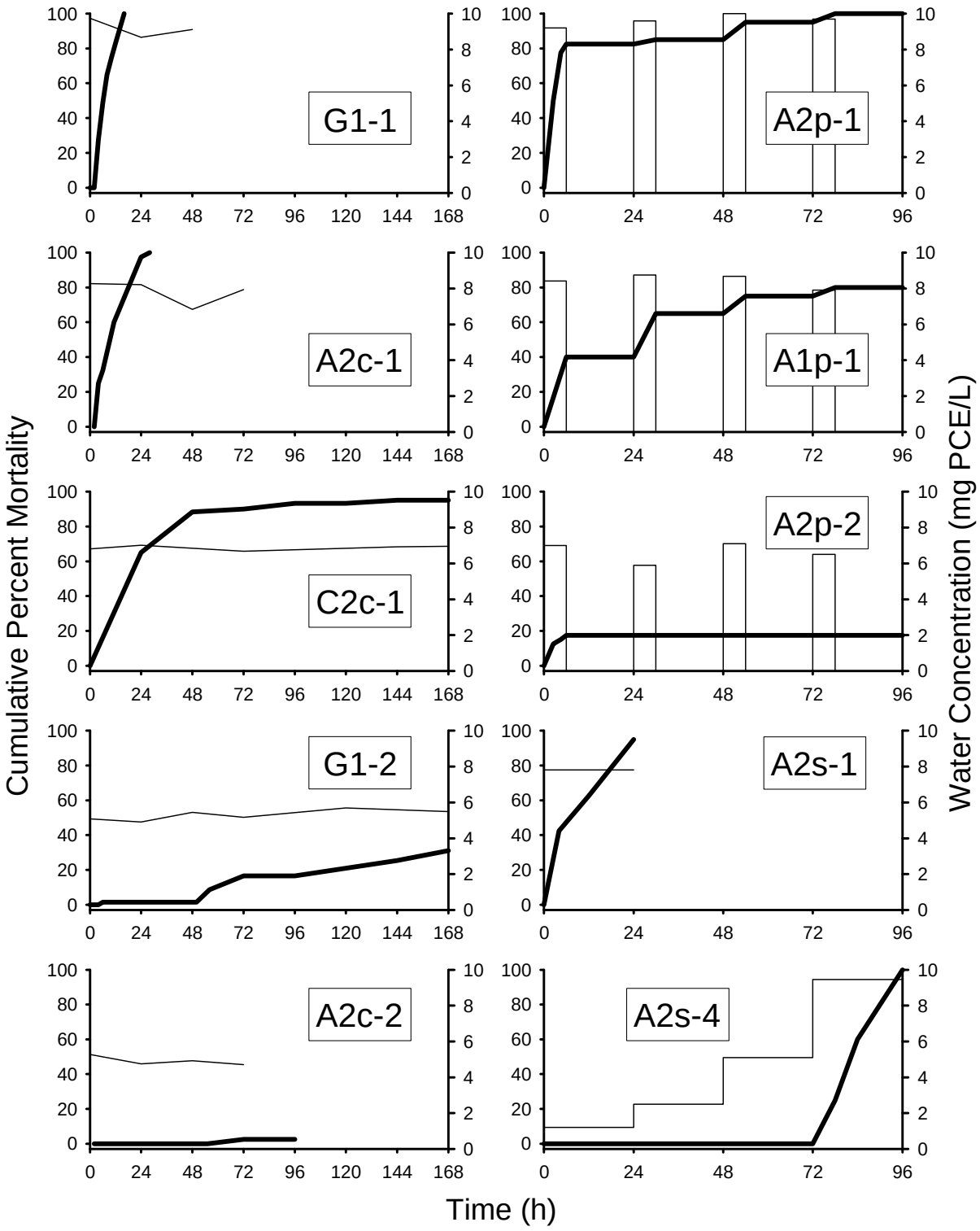
(3) 28-d tests of survival and growth, starting with 2- to 3-week-old fish. In Experiment C1, continuous exposure (Test C1c) and daily 6-h pulses (Test C1p6h) were evaluated. In Experiment C2, continuous exposure (Test C2c) was contrasted to exposures only during the first 2 weeks (Test C2b2w) or the final 2 weeks (Test C2f2w). In Experiment C3, continuous exposure (Test C3c), daily 3-h pulses (Test C3p3h), and daily 8-h pulses (Test C3p8h) were evaluated. In Experiment C4, continuous exposure (Test C4c) was contrasted with exposures of 1 d per week (Test C4p1d) and 3 d per week (Test C4p3d). The highest exposure concentrations in these tests was set to be near acutely lethal levels. Growth rates were determined by weighing random samples of test organisms at weekly intervals. Accumulation of PCE was measured in fish collected for growth determinations and, for experiments C3 and C4, in subsamples of fish that died.

(4) An experiment (Experiment G1) to determine the time dependence of growth effects, both during and after exposure. Fish were exposed to five levels of PCE for 7 d and then to clean water for 11 days. Growth rates were determined by weighing random samples of fish at 2-3 d intervals. Accumulation of PCE was measured in samples of fish collected for growth determination. Accumulation of PCE was also determined in fish dying at the higher exposure concentrations and in selected live fish collected throughout the period in which mortality occurred.

4.3 Mortality Observations

Figure 4.1 shows cumulative percentage mortality for five continuous exposure treatments from Experiments A2, G1, and C2 (left side) and five pulsed and stepped exposure treatments from Experiments A1 and A2 (right side). For Experiment G1, there was substantial sampling of surviving organisms throughout periods with high mortality rates. For such a situation, deaths during different observation intervals will have different effects on cumulative mortality depending on the number of live samples preceding each interval. Thus, cumulative

Figure 4.1. Observed mortality (bold lines) as a function of water concentration time-series (narrow lines) for selected continuous exposures from Tests A2, G1, and C2 (left side) and time-variable exposures from Tests A1 and A2 (right side).



percentage mortality at any time cannot be calculated simply as the sum of the mortalities observed up to that time divided by the starting number of organisms, even if this starting number is reduced by the live samples up to that time. Rather, for the "i^{th}" observation period, an incremental fraction mortality (f_{Mi}) is calculated as the deaths during that period divided by the actual number of organisms present at the start of the period. Then, the cumulative fraction mortality is calculated as $1-\Pi(1-f_{Mi})$ (i.e., the Kaplan-Meier estimator). Consider a test starting with 20 organisms in which 4 died during each of the first two observation intervals and 8 survivors were sampled at the end of the first observation interval. For the first period the incremental fraction mortality would be 0.20, based on the initial number of organisms. For the second period, the incremental fraction mortality would be 0.50, based on this period starting with 8 organisms after both the first period deaths and the sampling. The cumulative fraction mortality would be 0.60 after the second period, in contrast to 0.67 based simply dividing the cumulative deaths (8) by the starting number minus the live samples (20-8=12).

Figure 4.1 illustrates how mortality due to PCE has steep relationships to exposure concentration and time. For the continuous exposures (left panels of Figure 4.1), the highest concentration (Panel G1-1) resulted in complete mortality within 14 h, but just a 15% drop in the exposure concentration (A2c-1) resulted in complete mortality being delayed to 28 h, and an additional 15% drop (C2c-1) caused mortality to not reach 90% until 4 d and to not reach 100% within 7 d. Further reduction to about 5.0 mg PCE/L resulted in virtually no mortality over 96 h (Panel A2c-2) and 48 h (Panel G1-2), and for the latter exposure just slight increases in concentration after 48 h was enough to cause 30% mortality to be reached at 7 d. Although not shown in the figure, for continuous exposures in other experiments, mortality was never appreciable at exposures concentrations less than 5 mg PCE/L and was generally 100% within several days for exposure concentrations near and above 7 mg PCE/L.

The time variable exposures (right panels of Figure 4.1) also reveal steep relationships of mortality to time and concentration. For a 6-h pulse to previously unexposed fish, mortality exceeded 80% at 9.2 mg PCE/L (A2p-1), but was only 40% at 8.4 mg PCE/L (A1p-1), 12% at

7.0 mg PCE/L (A2p-2), and absent at 5 mg PCE/L (not shown). After the first 6-h pulse, there was no additional mortality for pulses of 6-7 mg PCE/L (Panel A2p-2), suggesting rapid reduction in chemical accumulation and/or stress between pulses, such that later pulses could not add enough stress to kill additional fish. Higher pulses (Panels A1p-1, A2p-1) did show some incremental mortality after the first pulse, suggesting that such recovery between pulses is not complete; however, this incremental mortality is also probably partly attributable to higher concentrations in the later pulses. Rapid recovery from toxic stress is also indicated by the absence of mortality during the intervals between the pulses.

For the stepped exposures, mortality was nonexistent when exposure was 5 mg PCE/L or less, and was 100% within 24 h once exposure was near or over 8 mg PCE/L (Panels A2s-1, A2s-4 in Figure 4-1). These stepped exposures also suggest prior exposure does not increase how rapidly mortality occurs once lethal exposures are imposed; rather, the opposite seems to be true. For A2s-4, despite the fact that the prior exposure should create body burdens more than halfway to lethal levels, mortality when the exposure is stepped up to over 9.5 mg PCE/L only reached 25% in 6 h, much lower than the 80% mortality for a 6-h pulse of similar concentration in Treatment A2p-1, and the 50% mortality for the first 6-h of a lower concentration (7.8 mg PCE/L) in Treatment A2s-1.

However, although the relationship of mortality to exposure concentration and time is obviously very steep, care should be taken not to infer too much about the exact relationships, because of uncertainties in exposure concentrations (due to limited sampling, measurement error, and/or time variability) and variability in organism susceptibility. Because even a 10-20% change in concentration appears to have substantial effects on mortality in some cases, analytical error of just the same amount can confound results and make inferences about differences between similar exposures uncertain. One example of possible uncertainties in exposure or variability among experiments is that in exposure A2p-1, mortality reaches 80% in the first 6-h pulse of 9.2 mg PCE/L, whereas only 50% mortality is reached in the first 6 h in exposure G1-1, where the measured concentration is actually slightly higher.

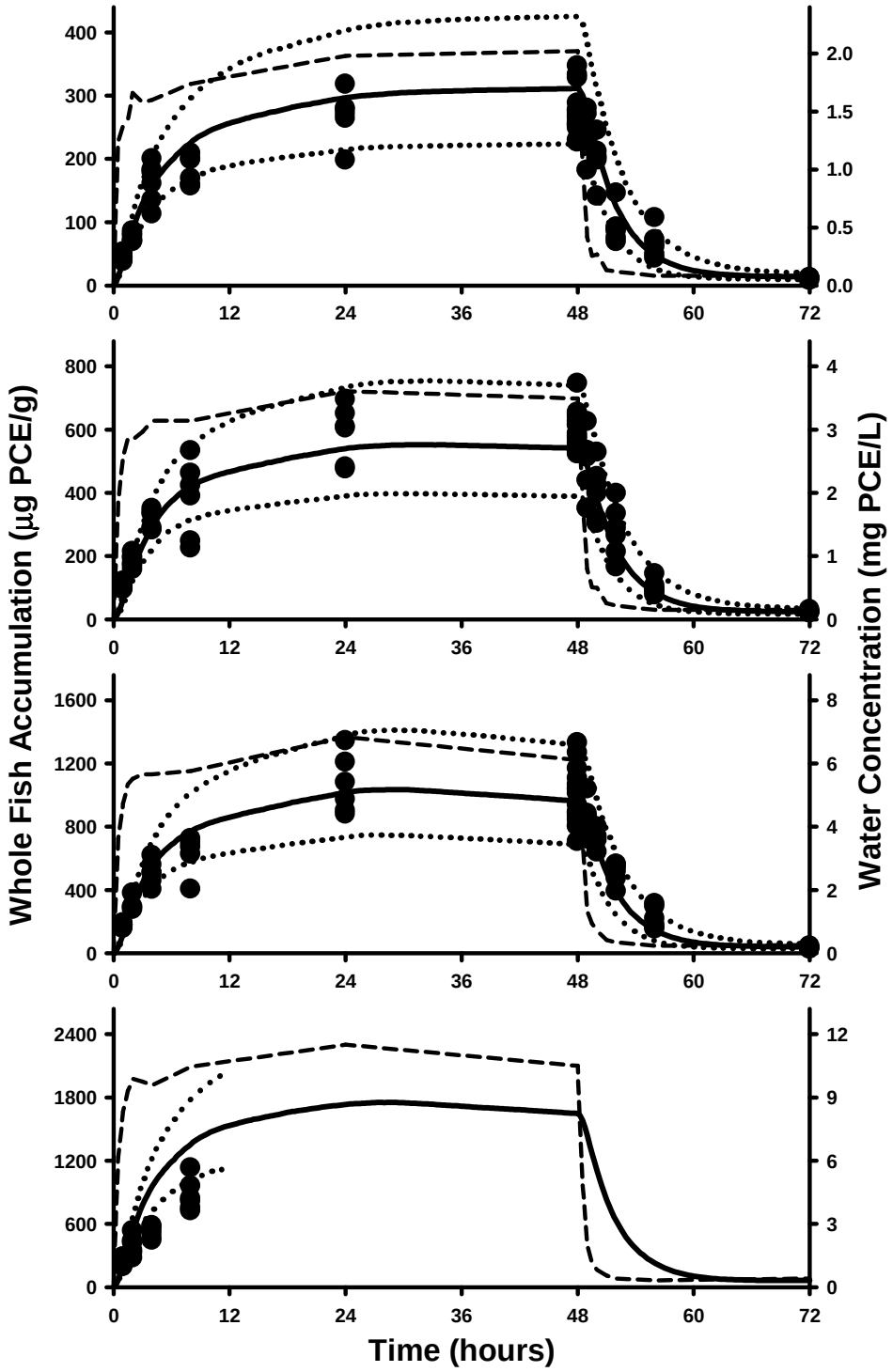
4.4 Kinetics of PCE Accumulation

Figure 4.2 shows the results for four treatments in the bioconcentration experiment (Experiment B1). For each treatment, the dashed line shows the water concentration, which rises within 2 h to at least 90% of its eventual values, and remains roughly constant until PCE input is terminated, after which the concentration shows an exponential decline, dropping about 90% in the subsequent 2 h. The PCE accumulations in sampled fish are shown by the filled circles. For the lower three concentrations, there is a rather rapid rise in the first 4 h to about half of the eventual value, and attainment of approximate steady state within 24 h. After the termination of exposure, accumulation declines similarly – by about half in 4 h and by more than 95% after 24 h. For the highest treatment concentration, fish exhibited toxic responses (lethargy, disequilibria) starting around 4 h and were all dead by 24 h. Because of this toxicity, accumulation rate relative to the treatment concentration was slower than in the lower, nonlethal treatment concentrations.

The simple, single-compartment, first-order accumulation model (Equations 2.1, 2.2) was parameterized based on the data from the lower three treatment concentrations in Figure 4.2. Uptake and elimination rate constants were both assumed to vary among individual fish with a log triangular distribution. Model parameters thus consisted of a mean and standard deviation for both $\log(k_U)$ and $\log(k_E)$. The accumulation model was integrated across the measured exposure times-series (using linear interpolation between data points) to provide, for each time at which accumulation was measured, a model-estimated accumulation as a function of model parameter values. Mathematical search routines (see Section 3) were then used to determine the parameter values that maximized the likelihood of the observed accumulations, resulting in estimated means and standard deviations of 1.619 and 0.226 for $\log(k_U)$ and -0.560 and 0.010 for $\log(k_E)$. This analysis thus inferred that the variability of the data was almost entirely due to variation in the uptake constant among individuals. These parameter values correspond to a median k_U of 41.6 ml/g/h and median k_E of 0.275/h.

Figure 4.2 also shows the fit of the model to the data, using the parameter estimates to

Figure 4.2. Measured accumulation (solid circles) versus time for a 48 h exposure and 24 h depuration at four concentration levels (dashed lines) in Test B1. Solid line denotes median prediction of single compartment, first order toxicokinetics model (median k_U=41.6 ml/g/h, median k_E=0.275/h) fitted to data from lower three concentration levels (highest level not used because of toxicity). Dashed lines denote 25th and 75th percentiles of predicted variation among individual organisms.



randomly generate 1000 pairs of k_U and k_E values and calculating the model-estimated accumulation for each pair of parameters. The bold lines denote the median of these accumulation estimates and the dotted lines denote the 25th and 75th percentiles.

For the lower three treatment concentrations, the median line is consistently near the middle of the data spread, except at 8 h into both the accumulation and elimination phases. This suggests the toxicokinetics are more complicated than the single-compartment, first-order behavior assumed in the model; for example, two-compartment kinetics would show slower uptake after several hours due to the outer compartment approaching equilibrium, subsequent uptake reflecting slower accumulation into an inner compartment (see Section 2.1.4). However, this deviation is relatively minor – at 8 h into accumulation, the deviation of the median model line from the median data averages less than 15% – and will not be further considered here.

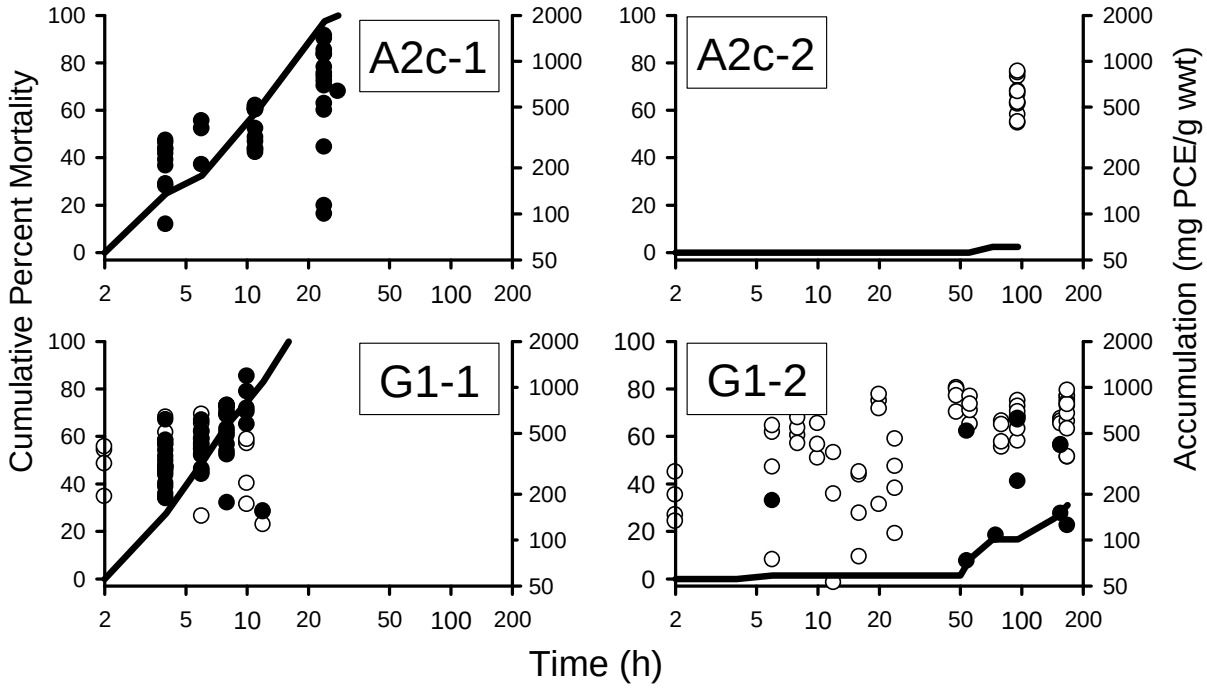
For the highest treatment concentration, the model overestimates accumulation, presumably due to the toxic effects on metabolic processes leading to reduced uptake. This illustrates a potential problem in incorporating such accumulation information into time-dependent effects models – exposures that are of interest because they cause effects will have different kinetics of accumulation than lower exposures which are often used for accumulation studies. To account for this aspect of toxicity, a reduction in the accumulation model parameters would be needed as a function of accumulation. Such possible refinements will not be made here but rather be a subject for possible later work as deemed appropriate.

4.5 Relationship of Mortality to PCE Accumulation

Figure 4.3 summarizes accumulation in both dead and surviving test organisms as a function of time and treatment concentration for two tests (A2c, G1) in which accumulation was comprehensively documented. It is assumed that accumulation in dead organisms did not change significantly between the times of death and sampling.

For Test A2c, at the highest concentration (Treatment A2c-1), mortality was complete in the first day and accumulation was measured in all the dead individuals (Figure 4.3). On average, accumulation increased with time-to-death, and at 24 h was especially variable. This

Figure 4.3 Measured PCE accumulation in dead (filled circles) and surviving (open circles) fish for two concentrations levels in Tests A2c and G1, contrasted with cumulative mortality (solid line).



trend and variability is not incompatible with toxicity models with a threshold lethal accumulation (e.g., Equations 2.1-2.7) because individuals with higher thresholds would on average take longer to reach their thresholds and individuals with low to moderate thresholds could still take long to reach these when their kinetic parameters are slower than average. However, the extremely large variability here is surprising given the steep mortality relationships that were observed (Figure 4.1), which suggests more similarity among individuals. This variability is also contradictory to the stochastic toxicity model (Equations 2.22-2.27), which assumes similarity among organisms.

Problems with applying these accumulation data to toxicity models explicitly based on accumulation become more evident given the data for the second highest concentration in Test A2c (Treatment A2c-2). Here, no individuals died, yet accumulation in a random sample of survivors had a higher mean and less variability than for the dead individuals in Treatment A2c-1 (Figure 4.3). This is fundamentally inconsistent with any toxicity model explicitly based on

whole-body accumulation. Given the distribution of accumulation observed for dead individuals in Treatment A2c-1, substantial mortality should have been observed in Treatment A2c-2.

Although not presented here, the lipid content of each fish was measured and the same problem – of lower PCE accumulation in dead organisms at a high exposure concentration than in surviving organisms exposed to a lower concentration – is observed for lipid-normalized whole-body accumulation values. This does not contradict the fundamental importance of accumulation to toxicity, but rather suggests that whole-body accumulation is not a good measure of the accumulation relevant to toxicity. A likely contributing factor to this problem is that toxicity should be related to specific compartments in the individual and accumulation in such compartments might have a different time course than whole-body accumulation. For example, PCE in the circulating blood and well-perfused tissues would respond more quickly to water concentrations, whereas total body accumulation, especially after substantial time, might mainly reflect chemical partitioned into toxicologically less important tissues. A more complicated toxicokinetic model and a toxicodynamics model focused on a specific compartment would thus be necessary to better relate mortality, accumulation, and exposure (e.g., Section 2.1.4), but such a model cannot be adequately supported with the data obtained in these experiments.

The results in Test G1 (Figure 4.3) reinforce these difficulties in relating accumulation to death in the toxicity models being considered. Although it must be acknowledged that some of the variability in this data is analytical uncertainty, there is clearly a large amount of variability among individuals, which is inconsistent with the stochastic model. But if just average accumulations are considered, the fact that the survivors in the later stages of Treatment G1-2 have accumulation greater than the individuals that died in this exposure also is incompatible with the tenets of the model. For the deterministic model, this is also a problem, because it is statistically improbable for the accumulation in the dead organisms to be so much lower than that of the randomly selected surviving organisms. Again, this does not belie the importance of accumulation, but rather only the utility of the whole-body accumulation monitored in these

experiments.

Despite these problems regarding the application of these accumulation measurements to the "explicit" forms of the toxicity models, these data can still be used to estimate model parameters, and thus be used to evaluate the implications of these problems to model predictions.

For the deterministic model, the desired parameters are the mean and variability of the distribution of lethal accumulation thresholds. This cannot simply be the mean and standard deviation of accumulation in dead organisms (unless the data set consists of exposures in which all the individuals died), because surviving organisms also have useful information in that they establish minimum values for their lethal accumulations. The data in Figure 4.3 were therefore subjected to maximum likelihood analyses to estimate the distribution of lethal accumulations, in which (a) the likelihood of the accumulation measured in a dead individual is the frequency of that accumulation value within the distribution and (b) the likelihood of the accumulation measured in a live individual is 1.0 minus the cumulative probability of that accumulation value within the distribution. For a pooled analysis of all the data in Figure 4.3, this resulted in a mean and standard deviation for the log(LA) of 2.79 and 0.39. Analyses on the separate tests produced similar values – 2.68 and 0.34 for Test A1c and 2.86 and 0.42 for Test G1. Analyses on just the treatments in which mortality was complete produced slightly lower means and standard deviations – 2.56 and 0.30 for Treatment A1c-1 and 2.65 and 0.22 for Treatment G1-1.

For the stochastic model, the toxicodynamic parameters to estimate from the accumulation data include the lethal accumulation threshold A_0 and the killing rate d (Equation 2.26). To estimate these parameters, it is necessary to first estimate, for the accumulation measured in each individual, the time-series of the accumulation leading up to that measurement, because this model uses the whole times-series to determine the integrated probability of death (Equations 2.22-2.26). As already noted, the fact that accumulation varies so much among individuals is inconsistent with the tenets of the stochastic model, but the desired times-series just depends on the value which was inferred (Section 4.4 above) to not be the source of this variability, so that the median value for k_e of 0.275/h could be used to generate these times-series

for illustrative purposes. Using such accumulation times-series for each individual, the probability of death can be determined as a function of A_0 and d, and a likelihood can thus be computed for the observed combination of dead and surviving organisms with their respective times and accumulations. However, the problems already noted in the accumulation data resulted in such an analysis inferring that this likelihood was maximized for a value for A_0 of 0 (no threshold for effects) with a value for *d* of 0.000027/h/(mg PCE/g wwt). Constraining A_0 to be 100 mg PCE/g wwt (lower than all but a few of the measured accumulations in dead organisms, Figure 4.3) and estimating just *d* resulted in a slightly higher value for d of 0.000032/h/(mg PCE/g wwt), which will be used in model calculations below.

4.6 Application of Implicit Deterministic Mortality Model

4.6.1 Model Parameterization

One consequence of both (a) the steep relationships of mortality to time and concentration and (b) the impact of exposure concentration uncertainties on interpreting these changes is that this data set does not have the information to support consideration of models of lethality more complex than the simplest ones in Section 2. In fact, this data set provides a good test of whether even the simplest model can be parameterized with limited information.

Mortality data from continuous exposures in Tests A2, C2, C3, C4, and G1 (in which the highest concentrations caused sufficient mortality to support model parameterization), were used to parameterize model D1 as described in Sections 2 and 3 of part A of this report, with no consideration being needed for how to treat delayed mortality, which was absent in these PCE tests. Parameters were estimated based on data from each individual test and on the pooled data from tests A1, C4, and G1 (in which mortality was monitored multiple times during the 12 h of exposure, whereas in tests C2 and C3 mortality was not monitored until 24 h). Table 4.1 summarizes the estimated parameter values from all six parameterizations.

The parameterization using the individual tests involves a small amount of information – often just two concentrations with mortality being complete in a short time at the higher concentration and being slight or absent at the lower concentration. Nevertheless, parameter

Table 4.1. Maximum likelihood estimates for deterministic model D1 parameters (implicit version). Parentheses denote standard error of estimate.

Tests Used for Parameter Estimation	Median Parameter Value		Mean $\log_{10}$ Parameter Value		Standard Deviation $\log_{10}$ Parameter Value	
	$LC\infty$ (mg PCE/L)	k (1/hr)	$LC\infty$	k	$LC\infty$	k
A2, G1, C4	6.15	0.160	0.789 (0.006)	-0.796 (0.027)	0.058 (0.003)	0.322 (0.021)
A2c	5.65	0.155	0.752	-0.810	0.020*	0.295
G1	5.84	0.141	0.766	-0.852	0.023	0.224
C2c	6.67	0.136	0.824	-0.862	0.020*	0.020*
C3c	6.65	0.104	0.823	-0.984	0.022	0.020*
C4c	6.05	0.167	0.782	-0.776	0.062	0.437

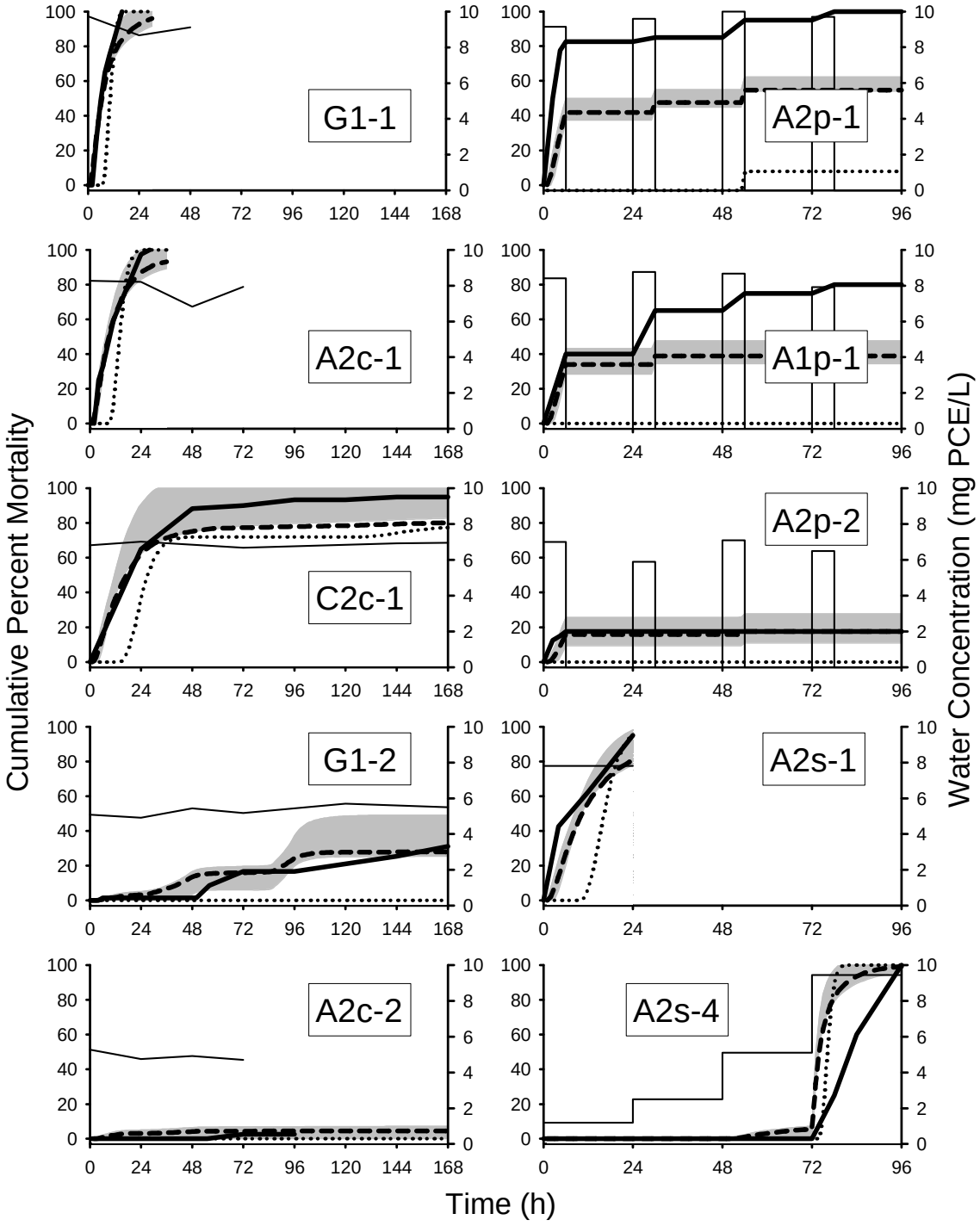
* minimum allowed log standard deviation = 0.02

estimates were successfully derived from each set, although for some sets a minimum variability among individuals was imposed on LC_∞ or k (this being a minimum standard deviation of the log parameter = 0.02, so that the maximum parameter value was at least 25% higher than the minimum). Despite the limited data, the median parameter estimates varied across the sets only from 5.65 to 6.05 mg PCE/L for LC_∞ and 0.141 to 0.167/h for k (Table 4.1) for the three data sets with early mortality information. For the tests without early mortality observations (C2c and C3c) parameter estimates were also close to these ranges, but were higher for the LC_∞ and lower for k_E, which raises the possibility that predicting mortality at short times will be uncertain based on such data sets.

4.6.2 Model Performance Using Parameterizations Based on Tests A2c, G1, and C4c.

For parameterizations based on tests in which early mortality observations were made (A2, G1, and C4), there were good fits to the mortality observed in the continuous exposures (left panels of Figure 4.4). For the high exposure concentrations (A2c-1 and G1-1), the model predicts rapid toxicity, including substantially more rapid toxicity when the exposure concentration is only slightly higher in G1-1 compared to A2c-1. The observed mortality falls

Figure 4.4. Observed mortality (bold solid lines) and predicted mortality as a function of water concentration time-series (narrow solid lines) for continuous exposures from Tests A2, G1, and C2 (left side) and time-variable exposures from Tests A1 and A2 (right side). All predictions are for the implicit deterministic model D1. Bold dashed lines denote pooled model parameterizations using tests A2c, G1, C4c and gray band denotes mean ± 1 std. dev. for separate model parameterizations using these three tests. Dotted lines denote mean of additional model parameterizations using 2 other tests without early mortality observations (C2c, C3c).



within 1.0 standard deviation of the mean prediction based on the individual parameterizations. On average, the model predicts the attainment of 90-100% mortality to be slower than observed, but uncertainties in such high mortality would be of little importance to risk assessments.

When exposure is reduced to about 7 mg PCE/L (C2c-1), the model predicts well the degree to which mortality slows down, with the observed mortality again bracketed by the uncertainty of predictions based on the individual parameterizations. For the pooled parameterization, the eventual degree of predicted mortality is 15% low, further illustrating the underestimation of high mortality already noted. This underestimation of mortality for the more tolerant individuals can occur due to overestimation of the variability of C_0 and/or k_E among individuals, resulting in a subset of organisms estimated to be more tolerant than appropriate. For the pooled parameterization, this can occur due to variation across experiments being interpreted by the model as variability across individuals.

Consistent with the observed mortality, model-estimated mortality for exposure G1-2 shows little mortality at early times when exposures are near 5 mg PCE/L and also shows moderate mortality as exposure lengthens and concentrations increase to about 5.5 mg PCE/L. For the parameterizations based on the individual data sets, substantial variability exists, but this is to be expected because the steepness of the response relationships causes small uncertainties in exposure concentrations and model parameters to have large effects on partial mortality predictions. For exposure A2c-2, the model predicts little or no mortality when exposure concentrations remain near 5 mg PCE/L, consistent with what was observed.

Large uncertainties of effects at concentrations in a steep section of the effects versus concentration curve are to be expected and are not generally of concern for risk assessments, for which the uncertainty of the concentration causing a particular effect is of more interest. With regard to this, the estimated effect concentration (EC) ranges across the different parameterizations are very small – 5.7 to 6.2 mg PCE/L for the 96-h EC_{50}, 5.0-5.4 mg PCE/L for the 96-h EC_{10}, and 5.9-6.6 for the 24-h EC_{50} – despite the limited data.

Although these good fits to the continuous exposures indicate that the model captures the

important features of the relationship of mortality to PCE concentration and exposure duration, model predictions for the pulsed and stepped exposures in the right panels of Figure 4.4 are also important for establishing the merits of the model. The model underestimates mortality for the most intense pulse exposure (A2p-1), even in the first pulse, by nearly a factor of 2.0. This is partly due to the underestimation already noted regarding the quickness of mortality for the more tolerant organisms, but also probably reflects the uncertainty issues also noted regarding steep toxicity relationships. For exposure A1p-1, the prediction for the first pulse is good, but the model fails to predict the observed incremental mortality in subsequent pulses. One possible reason for this is that the single kinetic constant in the model can cause an overestimation of the degree of recovery between pulses for this intense exposure (e.g., >90% reduction in accumulation and/or damage). In contrast, for the less intense pulse of A2p-2, the model does predict well both the degree of mortality in the first pulse and the absence of mortality in subsequent pulses.

Again, data presentations such as Figure 4.4 highlight uncertainties in effects at a particular exposure concentration, which are informative, but typically of less interest to risk assessments than the uncertainties in effect concentrations. For a single 6-h pulse, the average predicted EC50 is 9.8 mg PCE/L, whereas the observed EC50 is 8.5 mg PCE/L, just 13% lower. For four daily pulses, the observed EC50 is 7.5 mg PCE/L based on average pulse concentrations, 20% less than the average predicted EC50 of 9.4 mg PCE/L. Thus, the model underestimation of the risk of pulsed exposures is relatively minor.

For the stepped exposures, the good predictions for A2s-1 are expected because this is no different than the first day of the continuous exposures already discussed. The model does predict well that there will be total mortality when exposures are stepped up to lethal levels on later days after previous nonlethal exposures (A2s-4, and also A2s-2 and A2s-3, not shown, in which lethal exposures were on the second and third day). However, the slower rate of mortality in such incrementally increasing exposures, that appears to come from the prior nonlethal exposure, is not predicted. This suggests some importance of toxicokinetic or toxicodynamic

processes that are not included in the model. However, again, these have relatively little importance to the exposure concentrations predicted to be lethal for any particular exposure times-series shape.

4.6.3 Model Performance Using Parameterizations Based on Tests C2c and C3c.

When the model is parameterized based on tests in which early mortality is not monitored, predictions are worse. For continuous exposures with high concentrations, the average predicted mortality for parameterizations based on Tests C2 and C3 is delayed substantially from that observed (G1-1, A2c-1, C2c-1, A2s-1 in Figure 4.4). At the lower exposures of 5.0-5.6 mg PCE/L, the moderate mortality observed in Treatment G1-2 is not predicted at all, despite this occurring over a timeframe consistent with the observations in C2 and C3 used to parameterize the model. Little or no mortality is predicted for the pulsed exposures (A2p-1, A1p-1, A2p-2 in Figure 4.4). This emphasizes the need for at least some mortality information over a broad range of timeframes if these models are to be effective.

4.7 Application of Implicit Stochastic Mortality Model

4.7.1 Model Parameterization

As for the deterministic model, mortality data from continuous exposures in Tests A2c, C2c, C3c, C4c, and G1 were used to parameterize the implicit version of stochastic model S1, as previously described in Sections 2 and 3 in Part A of this report. Again, parameters were estimated based on data from each individual test and on the pooled data from Tests A1, C4, and G1. Table 4.2 summarizes the estimated parameter values from all six parameterizations. Although parameter estimates were successfully obtained, the estimates for k_E in four of the parameterizations were at a maximum allowed value (1.0/h, beyond which this parameter has no practical consequences), about 4-fold greater than the value estimated directly from bioaccumulation data in Section 4.4, and were more than 2-fold higher or lower in the other two parameterizations. This difficulty in the model parameterization reflects the fact that both k_E and d represent aspects of the kinetics of the toxicity response (k_E regards accumulation of chemical or damage and d addresses the mortality rate for a given accumulation), and the data must

Table 4.2. Maximum likelihood estimates for stochastic model S1 parameters (implicit version).

Tests Used for Parameter Estimation	Parameter Value			$\log_{10}$ Parameter Value		
	C_0	d	k_E	$C0$	d	k_E
A2, G1, C4	5.25	0.030	1.0*	0.718	-1.528	0.000*
A2c	5.14	0.040	1.0*	0.711	-1.401	0.000*
G1	5.28	0.040	0.604	0.723	-1.399	-0.219
C2c	6.35	0.054	1.0*	0.803	-1.270	0.000*
C3c	6.58	0.079	0.3*	0.719	-1.596	-0.898*
C4c	4.68	0.014	1.0*	0.670	-1.848	0.000*

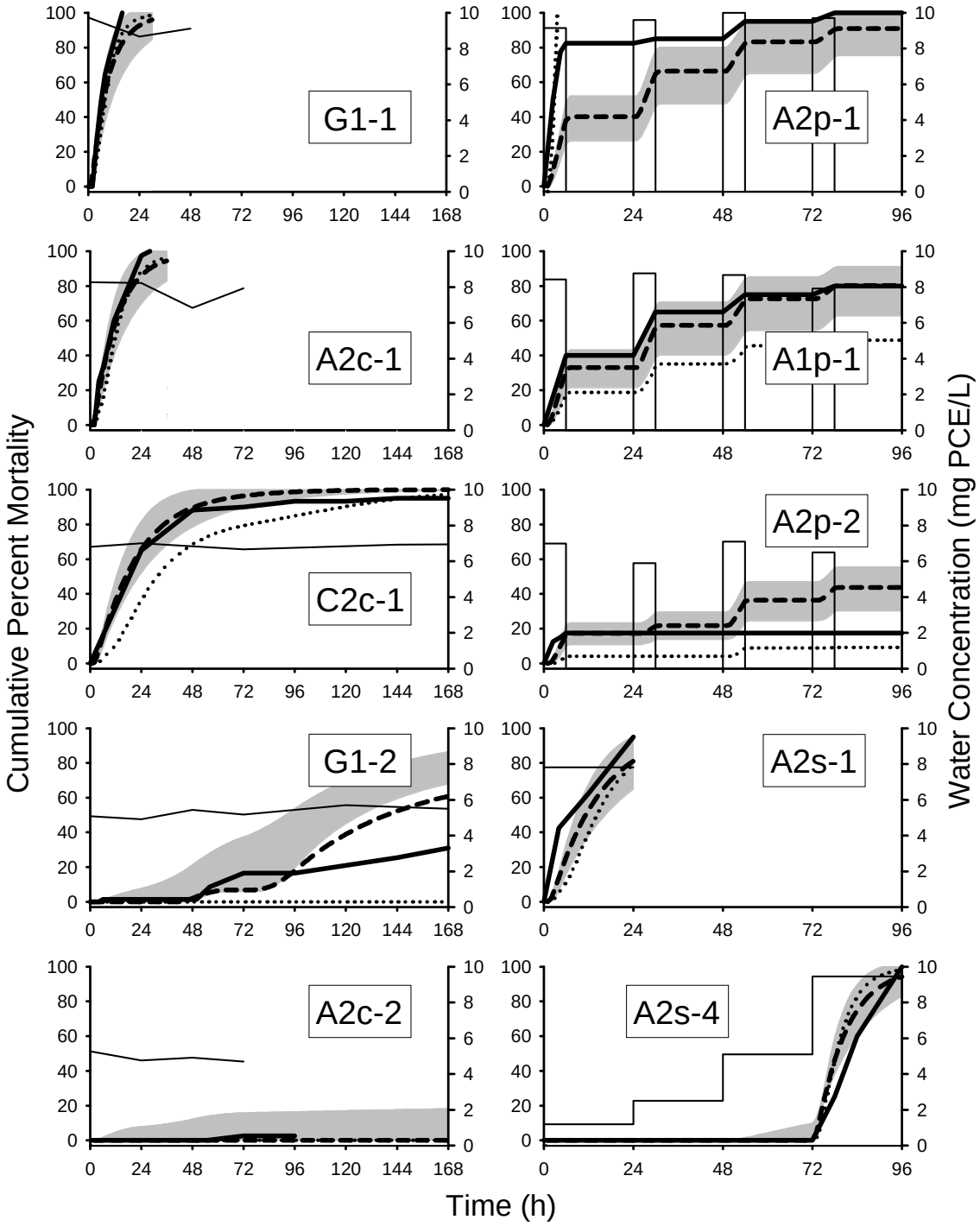
* log mean k constrained to be ≤ 0.0

contain at least some indication of two-phase kinetics for mortality for the model to partition the kinetics between these two processes. That k_E is so much greater than the known kinetics of accumulation is indicative of the model parameters not actually describing what they purport to.

4.7.2 Model Performance

When parameterized using Tests G1, A2c, and/or C4c, stochastic model mortality estimates show both limitations and merits of the model. As for the deterministic model, the rapid, high mortality in Treatments G1-1 and A2c-1 is predicted well (Figure 4.5), although with some underestimation of the death rate as mortality nears 100%. The model also predicts the slower and approximately complete toxicity for Treatment C2c-1. However, it greatly overestimates the mortality in Treatment G1-2. This is due to this model assuming no differences in sensitivity among individuals, so that once one individual dies, all the others will eventually die if exposure does not decrease below the threshold. Regarding this, the apparent leveling off of mortality for Treatment A2c-2 at the upper range of the predictions is due to such declining exposure. That this model lacks consideration of differences among organisms and treats partial mortality only as an issue of insufficient time to reach complete mortality is a limitation, and results in vanishingly small differences in ECs for different effect levels (an "all

Figure 4.5. Observed mortality (bold solid lines) and predicted mortality as a function of water concentration time-series (narrow solid lines) for continuous exposures from Tests A2, G1, and C2 (left side) and time-variable exposures from Tests A1 and A2 (right side). All predictions are for the implicit stochastic model S1. Bold dashed lines denote pooled model parameterizations using tests A2c, G1, C4c and gray band denotes mean ± 1 std. dev. for separate model parameterizations using these three tests. Dotted lines denote mean of additional model parameterizations using two other tests without early mortality observations (C2c, C3c).



or nothing" response) for prolonged exposures with constant, or regularly fluctuating, concentrations. However, for the PCE toxicity of concern here, this results in little error because the steep toxicity relationships of PCE already result in a narrow EC range. The ECs estimated using the stochastic model are similar to those for the deterministic model and do not vary much among the different parameterizations, being 5.3-5.5 mg PCE/L for the 96-h EC_{50}, 4.8-5.3 mg PCE/L for the 96-h EC_{10}, and 6.0-6.9 for the 24-h EC_{50}

Regarding the pulsed exposures, like the deterministic model, the stochastic model underestimates mortality in the first pulse at the high exposure in Treatment A2p-1. Unlike the deterministic model, the stochastic model does predict substantial incremental mortality across multiple pulses, which results in good predictions for Treatment A1p-1, but incorrectly predicts substantial mortality after the first pulse for Treatment A2p-2, where none was observed. Overall, this incremental mortality predicted by the stochastic model is of questionable merit because it reflects again that this model assumes all organisms have the same susceptibility and that the partial mortality in an initial pulse just reflects the finite probability within a finite time of any individual dying; thus, those individuals that survive the first pulse will eventually all succumb to subsequent pulses of sufficient magnitude. It thus does not allow for partial mortality that persists across pulses, as is evident in Treatment A2p-2 in Figure 4.5 and was also evident for copper in Section 3. However, this does not necessarily mean that this model does not provide mortality estimates that would still be useful in risk assessments. For a single 6-h pulse, the stochastic model estimates an EC_{50} (11.0 mg PCE/L) only 30% greater than what was observed (8.5 mg PCE/L), and, for four daily pulses, an EC_{50} of 6.9 mg PCE/L, within 10% of what was observed (7.5 mg PCE/L).

For the stepped exposures, the stochastic model successfully predicts that mortality will be heavy when exposure increases from about 5 mg PCE/L to about 9 mg PCE/L, and it better predicts the rate of mortality during the lethal step because the parameter *d* makes this rate less sensitive to prior exposure than for the deterministic model. However, like the deterministic model, it does not address how prior exposure might actually slow the mortality rate, as was

apparent in the data.

As for the deterministic model, in most cases, predictions are worse when the stochastic model is parameterized with tests lacking mortality observations at early times (Figure 4.5). This again emphasizes that these toxicity models require information on mortality across a reasonable broad range of time scale. Although the required data would require only modest additional effort in standard tests and already is often collected, this does create some problems in exploiting reported test results, which do not typically provide results across multiple exposure times, even when these data are collected.

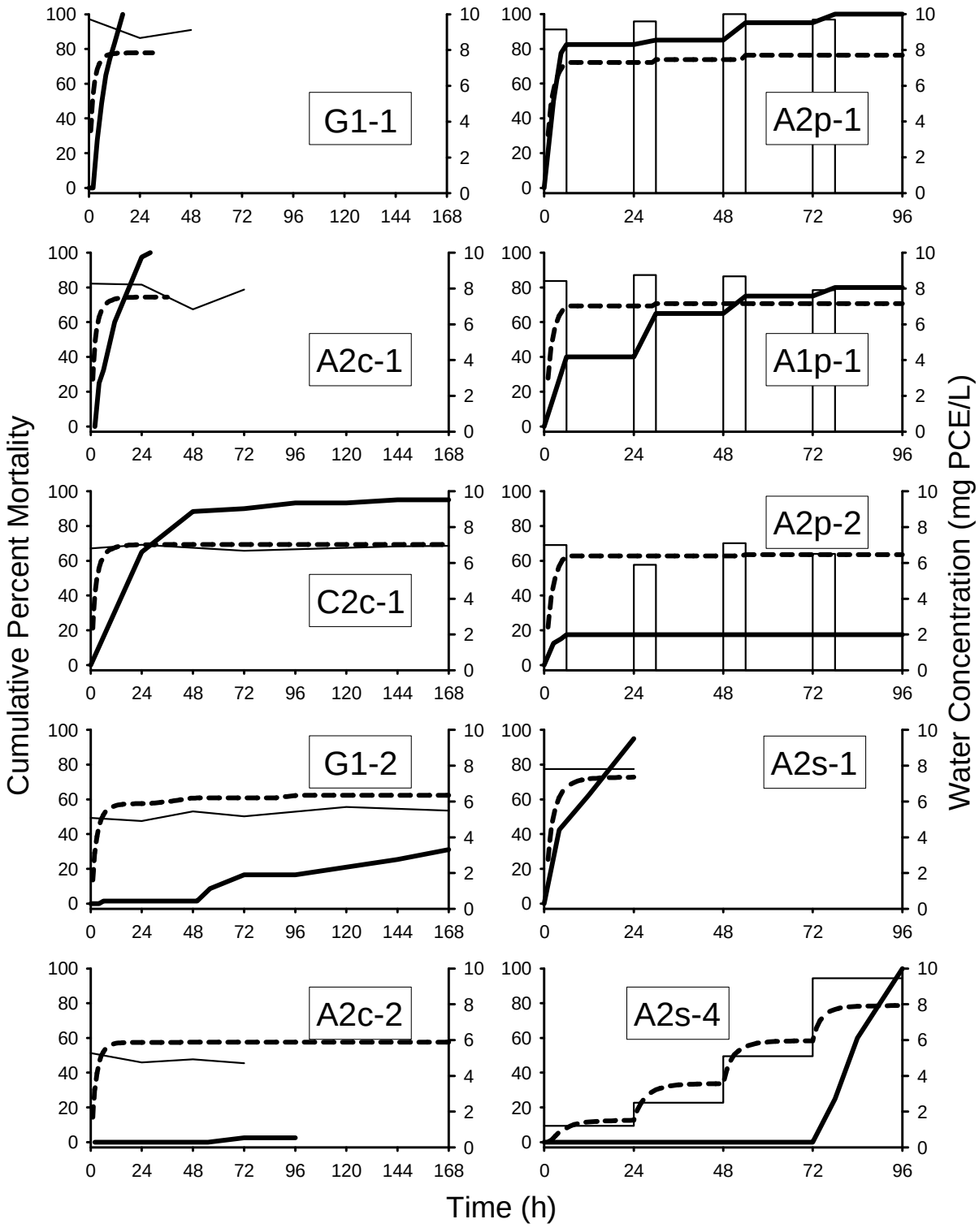
4.8 Application of Explicit Deterministic Mortality Model

The parameter estimates needed for application of the version of deterministic mortality model D1 that explicitly addresses accumulation were already reported in Sections 4.4 and 4.5. For toxicokinetics, the estimated means and standard deviations were 1.619 and 0.226 for $\log(k_U)$ and -0.560 and 0.010 for $\log(k_E)$, where the units of k_U are ml/g/h and those of k_E 1/h. For toxicodynamics, $\log(LA)$ was estimated based on the pooled data from Treatments A2c-1, A2c-2, G1-1, and G1-2 to have a mean 2.79 and a standard deviation of 0.39, where the units of *LA* are µg PCE/g wwt. With these parameter estimates, model predictions were made based on generating 1000 sets of values for k_U, k_E and *LA* and calculating fraction mortality as a function of time and exposure concentration based on 1000 model simulations using these sets.

The performance for this explicit implementation of the deterministic model is poor (Figure 4.6). For the continuous exposures, mortality is underestimated at high concentrations and overestimated at low concentrations. This poor performance is also apparent in the time-variable exposures on the right side of Figure 4.6, with mortality from pulsed Treatments A1p-1 and A2p-2 being overestimated. Problems with model estimates are particularly evident for Treatment A2s-4, where substantial mortality is predicted in the earlier steps, where no mortality was observed.

This poor performance reflects the problems noted earlier in Sections 4.4 and 4.5: specifically, the wide variability of model parameters resulting in predictions of a wide range of

Figure 4.6. Observed mortality (bold solid lines) and predicted mortality (bold dashed line) as a function of water concentration time-series (narrow solid lines) for continuous exposures from Tests A2, G1, and C2 (left side) and time-variable exposures from Tests A1 and A2 (right side). All predictions are for the explicit version of deterministic model D1 and use toxicokinetics parameters from Experiment B1 and lethal accumulations estimated from the pooled data of Tests A2c and G1.



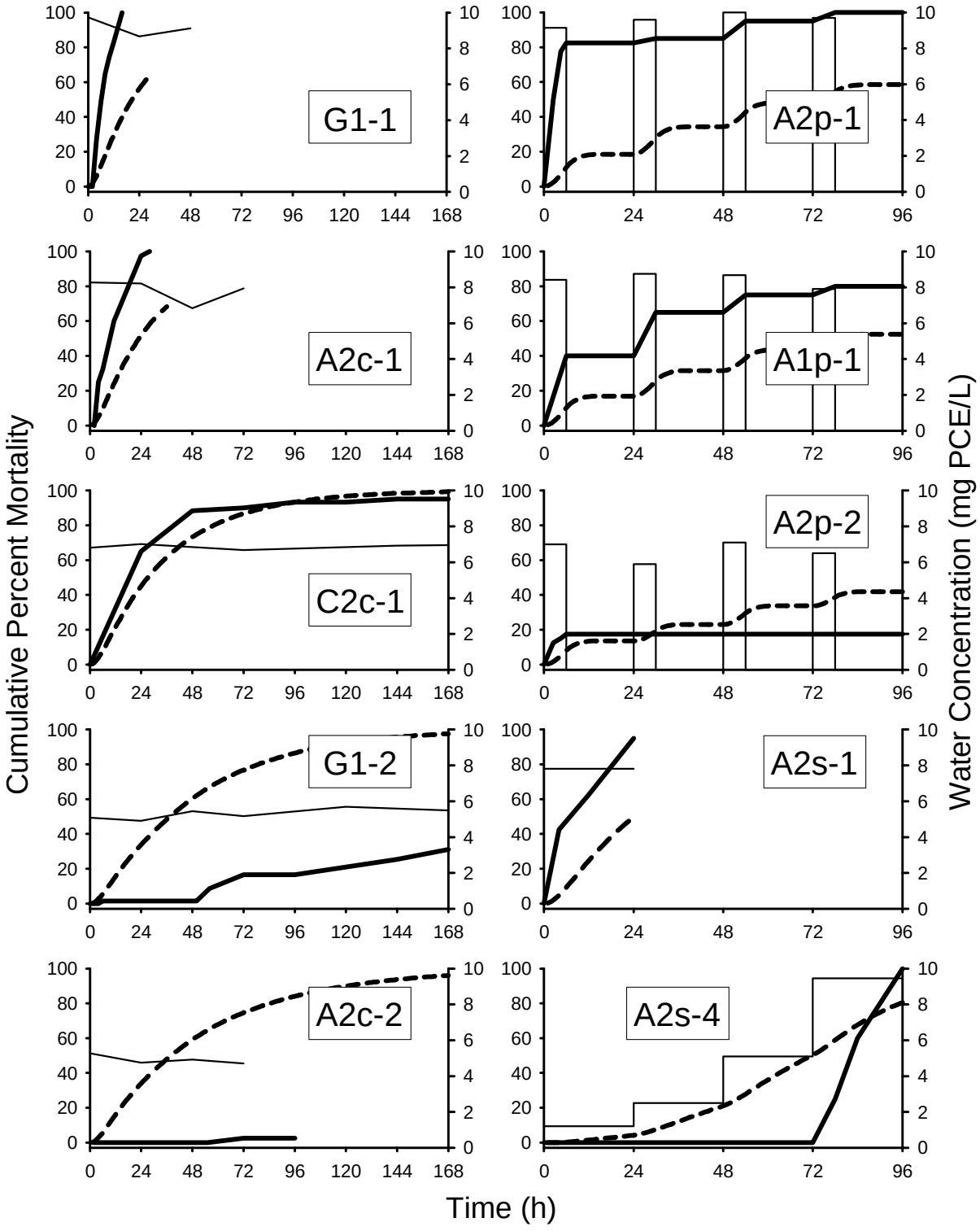
sensitivity of individuals to specific exposures, so that some individuals survive the high exposures and some succumb to lower exposures, contrary to what was observed. This does not belie the importance of accumulation to toxicity, but rather that these relationships are more complex than expressed in these models, and that this oversimplification can result in erroneous predictions, especially in the face of various uncertainties in parameterization data (e.g., accumulation measurements).

4.9 Application of Explicit Stochastic Mortality Model

The explicit form of the stochastic mortality model can be implemented based on the median k_U (41.6 ml/g/h) and k_E (0.275/h) estimated in Section 4.4, and the values specified in Section 4.5 of 100 μg PCE/g for the lethal accumulation threshold and 0.000032/h/(mg PCE/g wwt) for the killing rate *d*. Figure 4.7 provides the resultant model estimates of mortality time-series. Although the mortality patterns are much different than for the explicit deterministic model, there is again consistent underestimation of mortality for the higher exposures and overestimation for lower exposures.

The poor model performance again is due to problems already noted regarding the relationship of mortality to accumulation and the large variability among individuals in the measured accumulation and in the relationship of mortality to accumulation. As already noted, such variability is inherently inconsistent with the stochastic model. Using average parameter estimates based on variable data results in this model estimating mortality to occur at much lower concentrations than it actually does and for the mortality at high concentrations to occur more slowly than observed. Again, this does not refute the basic concepts of the model, but rather reflects errors that can occur when an overly simple model formulation is combined with uncertain data used for parameterization.

Figure 4.7. Observed mortality (bold solid lines) and predicted mortality (bold dashed lines) as a function of water concentration time-series (narrow solid lines) for continuous exposures from Tests A2, G1, and C2 (left side) and time-variable exposures from Tests A1 and A2 (right side). All predictions are for the explicit version of stochastic model S1 and use median toxicokinetics parameters from Experiment B1 and a killing rate estimated from pooled data of Tests A2c and G1, using a lethal accumulation threshold of 100 µg PCE/g wwt.



4.10 Summary and Implications to Aquatic Life Criteria

This analysis of mortality of juvenile fathead minnows exposed to pentachloroethane has further demonstrated that the toxicity models discussed in Section 2, when used in their "implicit" form and parameterized directly on the observed relationships between the time-series of mortality and continuous water exposure, can effectively describe these relationships and make useful predictions regarding time-variable exposures. These models are simple depictions of the toxicity processes and thus do not fully describe the mortality relationships. However, when direct data are lacking, they provide estimates for the effects of exposures that are accurate enough to be of use in aquatic life criteria applications and other aquatic risk assessments where information on the relationship of magnitude of effects to different exposure time-series is needed.

However, although these models rely on theoretical relationships of effects to accumulation, using versions of these models explicitly based on relationships of accumulation to exposure and mortality to accumulation did not provide good predictions for this case study. This was likely due to an oversimplification of the relationship of mortality to accumulation and to uncertainties and variability of data used in model parameterization. This does not argue against the importance of accumulation in toxicity relationships or in the utility of accumulation for more simple risk assessments. However, for predicting magnitude of toxicity across various exposure times-series, better models and data are needed.

It should finally be emphasized that the data and analyses of this section only relate to mortality in an acute timeframe and to toxicity mechanisms that operate with this timeframe. Actual criteria applications regarding even just mortality would need to also consider longer-term survival data, including the possibility of different mechanisms operating in different timeframes. Furthermore, the analyses here would be most relevant to highly variable exposure situations in which transient high exposures would make acute mortality relevant. For other exposures scenarios, chronic survival and other endpoints would be more of a concern. Subsequent reports in this series will be addressing longer exposures.

4.11 References

Erickson R, Kleiner C, Fiandt J, Highland T. 1991. Use of toxicity models to reduce uncertainty in aquatic hazard assessments: Effects of exposure conditions on pentachloroethane toxicity to fathead minnows. Internal report, U.S. Environmental Protection Agency, Mid-Continent Ecology Division, Duluth, MN, USA. 37 p.

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